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Evaluating the reducing properties of some antihypertensive and antibacterial drugs, through their reaction with iron (III) ions

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ABSTRACT

In this work the susceptibility of some antihypertensive and antibacterial drugs to oxidation were evaluated and compared through the application of a spectrophotometric method. Irbesartan (IRB), valsartan (VAL) and lisinopril (LSP) were selected as an example of antihypertensives, while two antibacterial drugs namely, cefixime (CFX) and cefprozil (CFP) were investigated. The applied method was based on the reaction of the cited drugs with iron (III) ions as an oxidizing agent. The resulted iron (II) ions reacted with o-phenanthroline forming the well-known highly stable orange-red colored chelate complex, which exhibits an absorption maximum at 510 nm. Different conditions were thoroughly studied and optimized. The reducing properties of the selected drugs were compared to each other (under the applied experimental conditions) and results show that these drugs act as reducing agents in the following order; CFP>CFX>IRB>LSP>VAL. The method was applied in a trial to investigate the stability of these drugs in air and results showed that cefprozil, cefixime and irbesartan are very labile to autoxidation.

KEYWORDS

Spectrophotometry;
Oxidation;
Reduction;
Irbesartan;
Valsartan;
Lisinopril;
Cefprozil;
Cefixime;
Ferric ions.

INTRODUCTION

Some drugs possess oxidizing properties others act as reducing compounds, thus the establishment of a simple method that gives an insight on the susceptibility of a certain drug towards oxidation is of great pharmaceutical values. Irbesartan[1] is designated as 2-butyl-3-{4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}-methyl)-1,3-diazaspiro[4.4] non-1-en-4-one and valsartan[1] as *N*-(1-oxopentyl)-*N*-{[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl}-*L*-valine 2 (Figure 1a,b). Irbesartan and valsartan are new antihypertensive drugs belonging to the family of angiotensin II receptor antagonists and lisinopril[1] is N^2-[(1*S*)-1-carboxy-3-phenylpropyl]-L-lysyl-L-proline (Figure 1c), it is a mem-ber of ACE inhibitors, or inhibitors of angiotensin-converting enzyme, they are indicated for treatment of high blood pressure, of congestive heart failure (CHF), and post-myocardial infarction (MI). Cefprozil[1] is designated as 7-[2-amino-2-(4-hydroxyphenyl)-acetyl]-amino-8-oxo-3-prop-1-enyl-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid and Cefixime[1] as (6*R*,7*R*)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2-carboxymetho-xyimino)acetyl]amino}-3-ethenyl-

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8-oxo-5-thia-1-zabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Figure 1d,e). Cefprozil and Cefixime are classified, respectively, as second and third generation cephalosporins, an important class of antibiotics that can be used to treat bronchitis, ear infections, skin infections, and other bacterial infections.

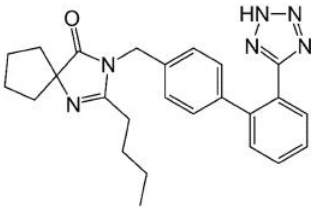

Figure 1a : Irbesartan

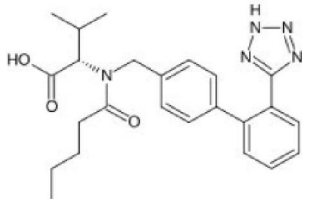

Figure 1b : Valsartan

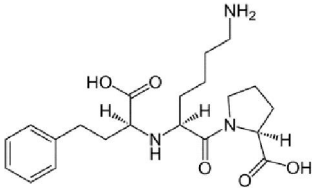

Figure 1c : Lisinopril

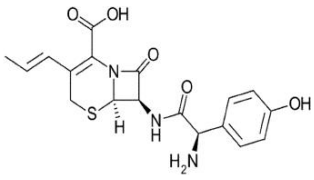

Figure 1d : Cefprozil

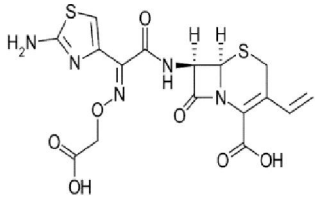

Figure 1e : Cefixime

Figure 1 : Chemical structure of the compounds under study

There is little spectroscopic analytical study on the selected drugs. The spectrophotometric methods were mainly developed for the deter-mination of the drugs in pure forms or in pharmaceutical formulations[2-30]. At present, no data are reported for evaluating and comparing the reducing power of the cited drugs. In this work, the tendency of the antihypertensive drugs irbesartan, valsartan and lisinopril and the two antibiotics cefprozil or cefixime towards oxidation was studied and compared using a spectrophotometric technique.

EXPERIMENTAL

Apparatus

A shimadzu 1601 spectrophotometer with quartz cells of 1-cm optical path length and a Hanna Microprocessor HI 9321 with a combined glass-saturated calomel electrode were used.

Materials and reagents

Anhydrous ferric chloride, o-phenanthroline (o-phen.) and ascorbic acid were supplied by Merck. All other solvents and reagents were of analytical-reagent grade. Double distilled water was always used.

The following antihypertensive standard powders and pharmaceu-tical formulations were analyzed: Irbesartan analytical standard and its tablets, Aprovel (Sanofi Co., Egypt) labeled to contain 300 mg per tablet, valsartan powder and its capsules, Disartan (Global Napi Co., Egypt) labeled to contain 160 mg per capsule, lisinopril standard powder and its tablets, Zestril (Astra Zenica Co., Egypt) labeled to contain 20 mg per tablet. The analyzed antibiotics: cefixime standard powder and its capsules, Ximacef (Sigma Co., Egypt) labeled to contain 400 mg per capsule and cefprozil standard powder and its tablets, Cefzil (Bristol-Myers-Squibb Co., Egypt) labeled to contain 500 mg per tablet.

Procedures

(a) Preparation of standards stock solutions

$2X10^{-3}$M standard drug solutions were prepared by dissolving the appropriate amount of pure drug in 95% ethanol for irbesartan and valsartan, in distilled water for lisinopril, in methanol for cefixime and in 5 ml methanol completed to 100-ml with distilled water, for cefprozil; sonication was applied whenever needed. The solutions were invariably prepared before use and whenever required dilute solutions were obtained by appropriate dilution with the same solvent.

Acetate buffer solutions (0.2 M) covering the pH range 2.5-5.5 were prepared by mixing 0.2 M acetic acid with 0.2 M sodium acetate. Iron (III) solution ($2x10^{-2}$ M) was obtained by dissolving an accurate amount of anhydrous ferric chloride in 20 ml distilled water, acidified with about 2 ml of concentrated hydrochloric acid. The pH of the solution was adjusted to 2.0 ± 0.1 using 1 M HCl or 1M NaOH before completed to 100 ml with water. The resulting solution was stored in a tight light-resistant container. $2x10^{-2}$ M solution of o-phenanthroline was prepared by dissolving an appropriate amount of the

compound in 5 ml of ethanol and diluted to 100 ml with water. Ascorbic acid solution ($2x10^{-4}$M) was freshly prepared and protected from air and light in a tight dark bottle.

(b) Reducing power assay procedure and construction of calibration graph

Aliquots of stock standard solution of drug corresponding to 42.8-214.20, 80.0-522.6, 26.4-88.3, 2.7-10.8 or 2.4- 9.7 g/ml IRB, VAL, LSP, CFX or CFP, respectively, were transferred into stoppered test-tubes, 0.5 ml of iron (III) solution ($2x10^{-2}$ M) and 2 ml o-phen. solution ($2x10^{-2}$ M) were added. The mixture was mixed by shaking and then heated on a boiling water-bath for 60 min for IRB and VAL or 40 min for LSP, CFX and CFP. After the heat treatment, the solution was immediately cooled to room temp-erature (25°C) using a cold water-bath and completed to volume in a 10-ml standard flask with acetate buffer pH 3.5. The absorbance was measured against a reagent blank prepared under the same conditions.

(c) Analysis of pharmaceutical formulations

The content of ten tablets or capsules were weighed and mixed; an accurately weighed portion of the powder equivalent to 10^{-3}M drug was transferred into a 100 ml beaker. Using a mechanical stirrer (or a sonicator) the powder was completely disintegrated in the same solvent system used for the preparation of standard stock solutions used for the construction of calibration graphs (except for lisinopril tablets, the powder was dissolved in a mixture of 80 % absolute ethanol and 20 % distilled water). The solution was filtered and the filtrate was made up to 50 ml with the corresponding solvent. An aliquot of the drug solution was analyzed as described under the assay procedure.

RESULTS AND DISCUSSION

In this report the reducing power of irbesartan, valsartan, lisinopril, cefixime and cefprozil were tested, this was carried out using ferric chloride as oxidizing agent. Oxidation of the investigated drugs with iron (III) yields Fe (II) ions, the amount of which is proportional to the drug concentration. The produced iron (II) ions form with o-phenantroline reagent a strongly orange-red colored complex of hexacovalent-type $(FeL_3)^{2+}$

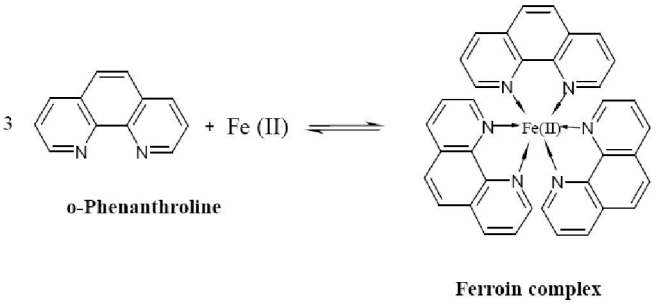

Figure 2 : The reaction of iron (II) ions with o-phenantroline forming a strongly orange-red colored complex of hexacovalent-type $(FeL_3)^{2+}$

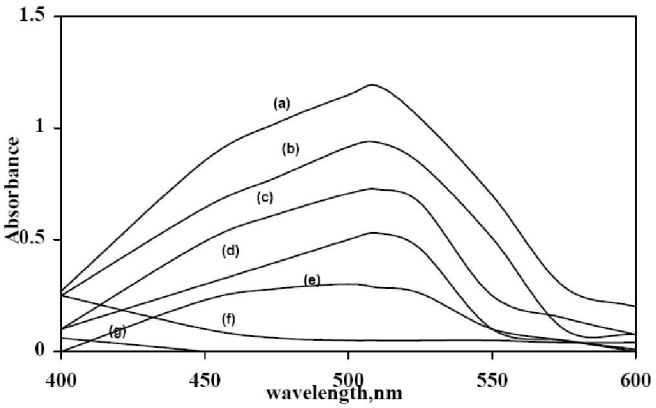

Figure 3 : Absorption spectra of the reaction products of 10^{-3} M Fe Cl_3, $3x\ 10^{-3}$ M o-phen. and drug : $2x10^{-6}$ M of CFP (a) or CFX (b), $5x10^{5}$ M IRB (c), 10^{-4} M VAL (d) or $2x10^{-5}$ M LSP (e). Blank solution in absence of drug (f); 10^{-3} M ferric chloride solution (g)

(Figure 2) which exhibits a maximum absorption band peak at 510 nm (Figure 3).

The solution of this colored chelate complex shows no change in color after many months [31]. The influence of different parameters on the color development was studied and the course of the reaction has been studied as a function of pH, concentration of reagents, temperature and heating time. It was found that the applied conditions of the assay procedure were the best for full color development.

Effect of pH

The effect of pH on the color intensity was studied over the pH range 2.5-5.5 in acetate buffer solutions (acidic medium is needed to prevent hydrolytic precipitation of Fe (III) and at more acidic medium the ferroin complex exhibits incomplete formation[31-33]. At pH range 3-4, the absorbance of the ferroin complex

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reached a maximum value, for all drug mixtures (Figure 4), cones-quently pH 3.5 value was chosen for completing the studies.

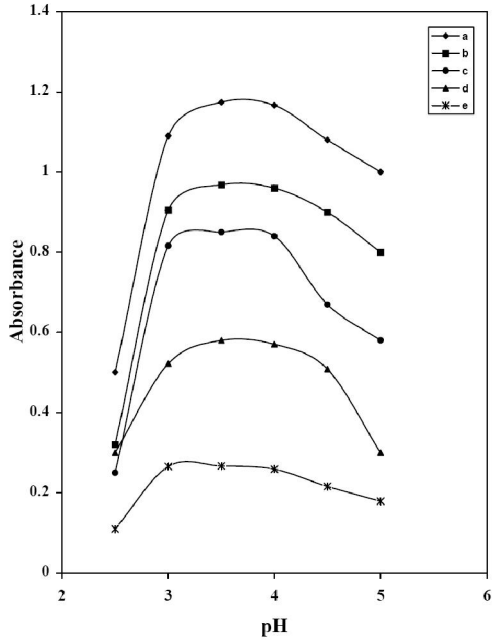

Figure 4 : Effect of pH on the absorbance of the reaction product of 10^{-3} M $FeCl_3$, 3x 10^{-3} M o-phen. and drug : 2x10^{-6} M of CFP (a) or CFX (b), 5x10^{5} M IRB (c), 10^{-4} M VAL (d) or 2x10^{-5} M LSP (e). λ = 510 nm

Effect of temperature and heating time on ferroin complex formation

This was carried out by following up the development of the color intensity of a mixture containing: 5x10^{-5} M of IRB, 10^{-4} M VAL, 2x10^{-5} M LSP or 2x10^{-6} M of either CFX or CFP; with 10^{-3} M of $FeCl_3$ and 3x10^{-3} M of 1,10-phenanthroline solution. After adding acetate buffer of the optimum pH the solutions were heated on a water bath thermostated at (50-100 °C) and heating time up to 70 min. It was found that heating the reactants to 100 °C for e" 50 min for IRB or VAL, and e" 30 min for LSP, CFX or CFP, was optimum to give maximum absorption intensity (Figure 5,6).

Effect of $FeCl_3$ concentration

0.2-1.2 ml of 2x10^{-2} M $FeCl_3$ solution were added to 1 ml of 5x10^{4} M of IRB, 10^{-3} M Val, 2x10^{-4} M LSP or 2x10^{-5} M of either CFX or CFP and 3ml of 2x10^{-2}M 1,10-phenanthroline in a total volume of 10 ml. The maximum absorbance was obtained after addition of about 0.4-0.7 ml ferric chloride solution. By adding more reagent a decrease in absorbance was observed (Figure 7). Thus, 0.5 ml of 2x10^{-2} M $FeCl_3$ solution was used in all measurements.

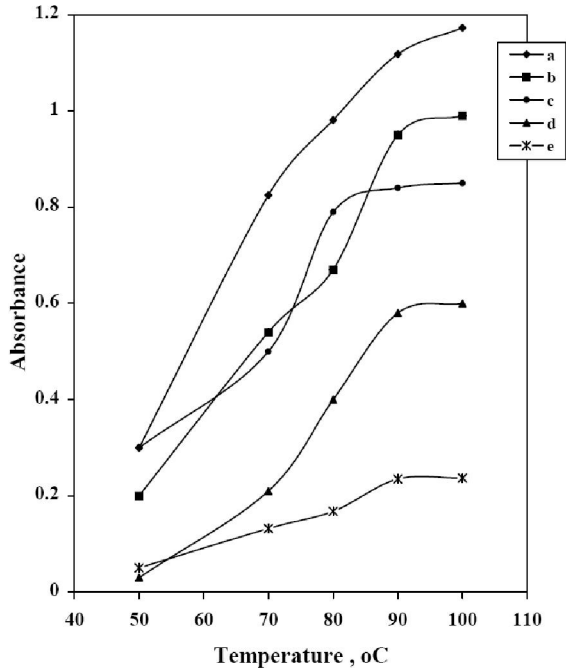

Figure 5 : Effect of temperature on the absorbance of the reaction product of 10^{-3} M $FeCl_3$, 3x 10^{-3} M o-phen. and drug : 2x10^{-6} M of CFP (a) or CFX (b), 5x10^{5} M IRB (c), 10^{-4} M VAL (d) or 2x10^{-5} M LSP (e). λ = 510 nm Heating time 40 min

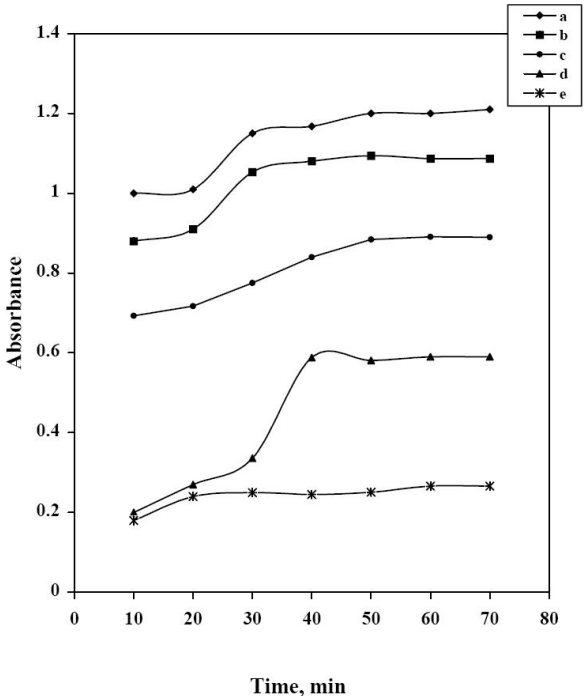

Figure 6 : Effect of heating time on the absorbance of the reaction product of 10^{-3} M $FeCl_3$, 3x 10^{-3} M o-phen. and drug : 2x10^{-6} M of CFP (a) or CFX (b), 5x10^{5} M IRB (c), 10^{-4} M VAL (d) or 2x10^{-5} M LSP (e). λ = 510 nm. Heating temperature 100 °C

Effect of 1,10- phenanthroline concentration

By changing the added volumes of $2x10^{-2}$ M of 1,10- phenanthroline to 1 ml $5x10^{4}$ M of IRB, 10^{-3} M Val, $2x10^{-4}$ M LSP or $2x10^{-5}$ M of either CFX or CFP and 0.5 ml of $2x\ 10^{-2}$ M $FeCl_3$ in a total volume of 10 ml. It was found that 1.5 ml of the phenanthroline reagent is sufficient for the production of maximum reproducible color intensity of the highly stable ferroin complex for all the studied drugs (Figure 8). Addition of more reagent did not affect color intensity; thus, 2 ml of $2x10^{-2}$ M o-phen. was used in the assay procedure and all other measurements.

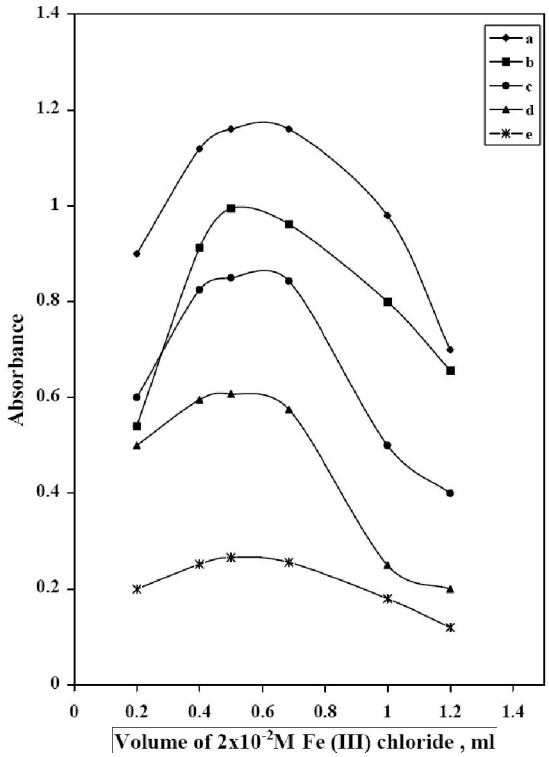

Figure 7 : Effect of volume of ferric chloride added on the absorbance of its reaction product with $3x\ 10^{-3}$ M o-phen. and drug : $2x10^{-6}$ M of CFP (a) or CFX (b), $5x10^{5}$ M IRB (c), 10^{-4} M VAL (d) or $2x10^{-5}$ M LSP (e). λ = 510 nm

The color of the formed complex was stable for more than 24 hours at room temperature (25 ± 5°C).

Quantification

Under the optimum conditions described, standard calibration curves were constructed by plotting absorbance versus concentration. **Beer's law** was obeyed and linear correlations were obtained in the ranges 4.28-21.42, 8.00-52.26, 2.64-8.83, 0.27-1.08 or 0.24- 0.97 mg/ml for IRB, VAL, LSP, CFX or CFP; respectively.

The molar absorpitivities, Sandell's sensitivity values, limits of detection and quantification were calculated and summarized in TABLE 1. The linearity was represented by regression equations and the correlation coefficients, as shown in TABLE 1, represent excellent linearity.

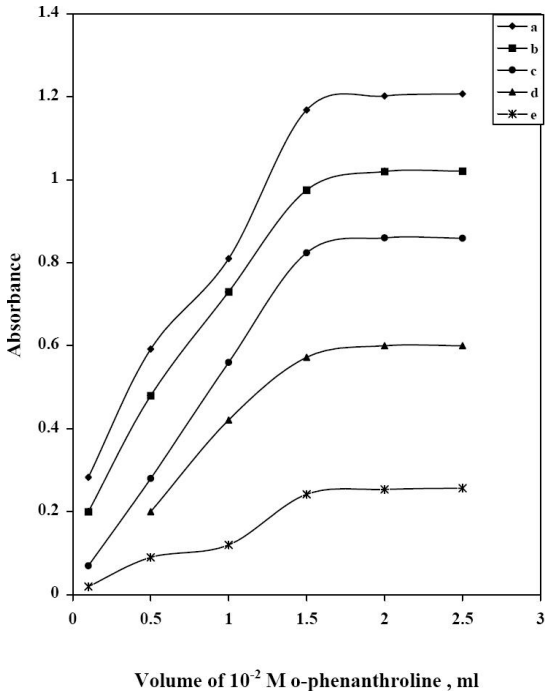

Figure 8 : Effect of volume of o-phenanthroline added on the absorbance of its reaction product with 10^{-3}M $FeCl_3$ and drug: $2x10^{-6}$ M of CFP (a) or CFX (b), $5x10^{5}$ M IRB (c), 10^{-4} M VAL (d) or $2x10^{-5}$ M LSP (e). λ = 510 nm

TABLE 1 : Optical characteristics

Parameter	IRB	VAL	LSP	CFX	CFP
Molar absorpitivity ($mol^{-1}cm^{-1}$)	1.68x104	$5.88x10^{3}$	$1.33x10^{4}$	$4.57x10^{5}$	$5.83x10^{5}$
Sandell's sensitivity $x10^{3}$ (μg cm^{-2})	25.42	73.96	33.12	0.98	0.70
Correlation coefficient (r)	0.9998	0.9981	0.9995	0.9997	0.9998
Regression equation (Y*) Slope (b)	0.04	0.015	0.047	0.97	1.46
Intercept (a)	-0.041	-0.050	-0.056	0.034	0.02
Detection limit (DL) μg ml^{-1}	0.475	1.405	0.565	0.037	0.0115
Quantitation limit (QL)	1.584	4,685	1.885	0.123	0.038

***Y= a+ bx, where x is the concentration in ìg ml^{-1}**

Accuracy and precision

The results of the intra-day and inter-day accuracy and precision of the method are summarized in TABLE 2 and TABLE 3. The inter-day and intra-day precisions were examined by analysis of each drug at three different concentrations each five times a day for three

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consecutive days. The precision of the proposed method is fairly high, as indicated by the low values of SD and % RSD respectively. In addition, the inter-day and intra-day accuracy was proved by the low values of % Er. The analytical results for accuracy and precision show that the methods proposed have good repeatability and reproducibility.

TABLE 2 : Intra-day precision

Conc added (µg/ml)	Intra-day precision			
	Conc found ± SD (µg/ml)	RSD%	ER%	Recovery %
Irbesartan				
5	4.980±0.015	0.301	-0.400	99.60
10	10.062±0.112	1.113	0.620	100.62
15	15.101±0.125	0.828	0.673	100.67
Valsartan				
10	10.011±0.123	1.229	0.110	100.11
25	24.953±0.131	0.525	-0.188	99.81
40	39.970±0.146	0.365	-0.075	99.92
Lisinopril				
4	3.992±0.080	2.000	-0.200	99.80
6	5.981±0.071	1.187	-0.317	99.68
8	8.024±0.101	1.259	0.300	100.30
Cefixime				
0.3	0.304±0.001	0.329	1.333	101.33
0.7	0.695±0.008	1.151	-0.714	99.28
1.0	1.016±0.004	0.394	1.600	101.60
Cefprozil				
0.3	0.297±0.005	1.683	-1.000	99.00
0.5	0.510±0.003	0.588	2.000	102.00
0.8	0.794±0.006	0.756	-0.750	99.25

Reducing properties evaluation

Molar absorptivities of the selected drugs were compared to each other (under the applied experimental conditions) and results (TABLE 1) show that these drugs act as reducing agents in the following order; CFP>CFX>IRB>LSP>VAL. The same method (2.3.2) was applied to 1ml of $2x10^{-4}$ M drug or to the same concentration of the strong reducing agent ascorbic acid with keeping the reaction mixture at 37°C for 30 min. (due to rapid decomposition of ascorbic acid at high temperature). Increased absorbance of the two antibiotics mixtures than that of ascorbic acid indicates stronger reducing power (TABLE 4). Moreover, the stability of the investigated drugs on exposure to air was tested. For this purpose, a thin layer of drug was spreaded in a petri-dish and exposed to air (in dark) for two weeks at 30 ± 2 °C and relative humidity (RH: 50-70%), a parallel set was kept under similar conditions but protected from air. On applying the proposed method to the two sets of drugs, a distinct decrease in the recovery % was observed for the two antibiotics (Table 5), indicating occurrence of a loss in the reducing activity of the two drugs due to exposure to air. Results show that irbesartan is also labile to autoxidation while no change was observed for either lisinopril or valsartan. All results confirm the powerful reducing character of cefixime and cefprozil, consequently they can have antioxidant properties. Moreover, adequate air protection should be carried.

TABLE 3 : Inter-day precision

Conc Added (µg/ml)	Inter-day precision			
	Conc found ± SD(µg/ml)	RSD%	ER%	Recovery %
Irbesartan				
5	5.089 ±0.07 1	1.395	1.780	101.78
10	10.171±0.132	1.298	1.710	101.71
15	15.085±0.146	0.968	0.567	100.56
Valsartan				
10	10.032±0.141	1.406	0.320	100.32
25	25.044±0.189	0.755	0.176	100.17
40	40.023±0.293	0.732	0.0575	100.06
Lisinopril				
4	4.062±0.053	1.305	1.550	101.55
6	6.031±0.094	1.559	0.517	100.52
8	8.052±0.120	1.490	0.650	100.65
Cefixime				
0.3	0.298±0.004	1.342	-0.667	99.33
0.7	0.705±0.009	1.277	0.714	100.71
1.0	1.016±0.011	1.083	1.600	101.60
Cefprozil				
0.3	0.298±0.005	1.677	-0.667	99.33
0.5	0.503±0.008	1.590	0.600	100.60
0.8	0.806±0.013	1.613	0.750	100.75

TABLE 4 : Comparative results of absorbencies of the selected drugs to ascorbic acid (keeping the reaction mixture at 37°C for 30 min)

Drug ($2x10^{-5}$M)	IRB	VAL	LSP	CFX	CFP	Ascorbic acid
Absorbance	0.23	0.01	0. 04	0.765	0.725	0.717

The two antibiotics contain one or more sulphide group in their molecules (Figure 1d,e), which can be

oxidized fairly readily to the sulphoxide forms (R_2SO) and further to the sulphone forms (R_2SO_2)[34].

TABLE 5 : Analysis of irbesartan, valsartan, lisinopril, cefixime and cefprozil in absence and in presence of air

Drug*	Recovery (%) **	
	In absence of air	In presence of air
IRB	100.13	91.74
VAL	100.35	100.21
LSP	101.15	100.06
CFX	99.84	58.95
CFP	100.55	40.26

*** Drug concentration = 8µg ml^{-1} (IRB, VAL and LSP), 0.5 µg ml^{-1} (CFX and CFP); **The results are the mean of three determinations**

TABLE 6 : Determination of irbesartan, valsartan, lisinopril, cefixime and cefprozil in their pharmaceutical formulations

Drug	Name of preparation	conc taken µg/ml	conc found* µg g/ml	Recovery ± SD %
IRB	Aprovel (300mg/tab)	10	9.950	99.50± 0.72
VAL	Disartan (160mg/cap)	25	24.870	99.48 1±.0.5
LSP	Zestril (20mg/tab)	6	5.980	99.66 ± 0.59
CFX	Ximacef (400mg/cap)	0.7	0.695	99.28± 0.58
CFP	Cefzil (500mg/tab)	0.5	0.497	99.40±0.46

*** Average of five determinations**

Determination of irbesartan, valsartan, lisinopril, cefixime and cefprozil in their pharmaceutical formulations

The method was successfully applied to the determination of the drugs in pharmaceutical formulations (capsules or tablets). The results are summarized in TABLE 6. The recoveries percent and the standard deviations (average of five determinations) are within the range 99.28-99.66% and 0.46-0.72, respectively, reflecting a satisfactory degree of accuracy of the proposed method.

CONCLUSION

From this study, it is obvious that the selected drugs act as reducing agents in the order CFP>CFX>IRB>LSP>VAL. The study shows also the two antibiotics, cefixime and cefprozil possess higher reducing power than that of ascorbic acid (and thus can be classified as strong reducing agents). Stability study of the pure drugs in presence of air shows the susceptibility of cefprozil, cefixime and irbesartan to oxidation by atmospheric oxygen, consequently, adequate air protection should be adopted for their packaging storage and handling. The method was also, successfully applied to the determination of the drugs in pure forms and in pharmaceutical formulations (capsules or tablets).

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